ZBTB17 (MIZ1) Is Important for the Cardiac Stress Response and a Novel Candidate Gene for Cardiomyopathy and Heart Failure

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Abstract: BACKGROUND Mutations in sarcomeric and cytoskeletal proteins are a major cause of hereditary cardiomyopathies, but our knowledge remains incomplete as to how the genetic defects execute their effects. METHODS AND RESULTS We used cysteine and glycine-rich protein 3, a known cardiomyopathy gene, in a yeast 2-hybrid screen and identified zinc-finger and BTB domain-containing protein 17 (ZBTB17) as a novel interacting partner. ZBTB17 is a transcription factor that contains the peak association signal (rs10927875) at the replicated 1p36 cardiomyopathy locus. ZBTB17 expression protected cardiac myocytes from apoptosis in vitro and in a mouse model with cardiac myocyte-specific deletion of Zbtb17, which develops cardiomyopathy and fibrosis after biomechanical stress. ZBTB17 also regulated cardiac myocyte hypertrophy in vitro and in vivo in a calcineurin-dependent manner. CONCLUSIONS We revealed new functions for ZBTB17 in the heart, a transcription factor that may play a role as a novel cardiomyopathy gene.

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Original Article

ZBTB17 (*MIZ1*) Is Important for the Cardiac Stress Response and a Novel Candidate Gene for Cardiomyopathy and Heart Failure

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Background—Mutations in sarcomeric and cytoskeletal proteins are a major cause of hereditary cardiomyopathies, but our knowledge remains incomplete as to how the genetic defects execute their effects.

Methods and Results—We used cysteine and glycine-rich protein 3, a known cardiomyopathy gene, in a yeast 2-hybrid screen and identified zinc-finger and BTB domain-containing protein 17 (*ZBTB17*) as a novel interacting partner. *ZBTB17* is a transcription factor that contains the peak association signal (rs10927875) at the replicated 1p36 cardiomyopathy locus. *ZBTB17* expression protected cardiac myocytes from apoptosis in vitro and in a mouse model with cardiac myocyte–specific deletion of *Zbtb17*, which develops cardiomyopathy and fibrosis after biomechanical stress. *ZBTB17* also regulated cardiac myocyte hypertrophy in vitro and in vivo in a calcineurin-dependent manner.

Conclusions—We revealed new functions for *ZBTB17* in the heart, a transcription factor that may play a role as a novel cardiomyopathy gene. **(*Circ Cardiovasc Genet*. 2015;8:643-652. DOI: 10.1161/CIRCGENETICS.113.000690.)**

Key Words: cardiomyopathies ■ genetics ■ heart failure ■ models, animal ■ mutation

Mutations in >50 genes, mostly encoding sarcomeric and Z disc proteins, cause hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) that lead to heart failure.[1] Genetic variation in a Z disc gene can cause various forms of cardiomyopathies such as HCM or DCM perhaps because of pleiotropic effects on survival and hypertrophic pathways.[2] The muscle LIM protein (cysteine and glycine-rich protein 3 [CSRP3]) is a Z disc protein involved in cardiac mechanosensation that is important for myocyte-specific survival pathways through interactions with other Z disc proteins.[3–5] *CSRP3* mutations have been found in both HCM and DCM patients,[3,4,6] and we used protein–protein interaction studies to identify new CSRP3-interacting partners that may be important for cardiac myocyte survival or cardiomyopathy.

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Methods

All primers and antibodies used are listed in Tables I to III in the Data Supplement. Tissue culture experiments were performed using primary neonatal and adult rat cardiac myocytes, and animal experiments were performed using conditional knockout (cKO-Zbtb17), overexpressing transgenic (TG-ZBTB17) and double-transgenic

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†Connie Pfeiffer passed away in 2013. We dedicate this work to her.

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(TG-ZBTB17/KO-Ppp3cb) protein phosphatase 3, catalytic subunit, β isozyme (calcineurin Aβ, Ppp3cb) animals.

The procedures in animal studies have been followed in accordance with institutional guidelines. Human studies have been approved by an institutional review committee, and that the subjects gave informed consent.

Yeast 2-hybrid, Western blot, quantitative real-time polymerase chain reaction, and other assays were performed as described elsewhere.[3–5,7] Statistical evaluations were performed by nonparametric Mann–Whitney *U* tests and unpaired Student *t* tests (more details are available in the Data Supplement).

Results

CSRP3 Interacts With ZBTB17

Yeast 2-hybrid screens using CSRP3 as bait[3] classified α-actinin and telethonin as CSRP3-interacting proteins that are important determinants of cardiac function[5,8] and also identified ZBTB17 (Figure 1A). ZBTB17 is a member of the poxvirus and zinc-finger (POZ or BTB) domain/zinc-finger transcription factor family with no previously described role in the heart.[9] We confirmed the CSRP3–ZBTB17 interaction by coimmunoprecipitation in vivo (Figure 1B), cross-linking of recombinant proteins (Figure 1C), and immunohistochemistry studies (Figure 1D; Figure I in the Data Supplement). These data identify ZBTB17 as a novel CSRP3-interacting protein.

Analysis of a Human ZBTB17 Variant (T106M)

ZBTB17 is encoded on human chromosome 1 at the replicated 1p36 cardiomyopathy locus.[10–12] The peak DCM association at this locus (rs10927875; $P=1.3\times10^{-7}$)[12] is located in intron 2 of *ZBTB17*, with further evidence of association of this single nucleotide polymorphism with DCM in Chinese population.[12,13]

Considering rare polymorphisms (allele frequency <0.1%; population prevalence of cardiomyopathies ≈1:500[1]), we identified a p.T106M variant in the Exome Variant Server. The p.T106M variant could not be stably expressed in cardiac myocytes (Figure 2A), bound poorly to CSRP3 (Figure 2B), caused structural abnormalities to the POZ domain (Figure 2C and 2D), and may represent a rare haploinsufficient polymorphism. Together, these data point to *ZBTB17* as a possible disease gene at the 1p36 cardiomyopathy locus and suggest a role for a transcription factor in heart failure, as recently shown for left ventricular non compaction cardiomyopathy.[14]

Analysis of ZBTB17 Function In Vitro

To study function in vitro, we overexpressed *ZBTB17* in cardiac myocytes and found that it induced hypertrophy (Figure 3A and 3B; Figure IIA in the Data Supplement). CSRP3 and other CSRP3-interacting proteins have important roles in cardiac myocyte survival, and this was also observed for ZBTB17 (Figure 3C; Figure IIB in the Data Supplement). Calcineurin activity is of central importance for prohypertrophic signaling and myocyte survival,[15,16] and in both the hypertrophy and the cell death experiments inhibition of calcineurin attenuated the effects of ZBTB17, suggesting an interaction (Figure 3A and 3C). Hence, ZBTB17 regulates cardiac hypertrophy and cell survival, a recognized dual-property of genes that are of central importance for cardiac myocyte biology.[15–18]

Analysis of ZBTB17 Function In Vivo

Mice with global deletion of *Zbtb17* die because of a gastrulation defect.[19] Therefore, we crossed floxed *Zbtb17* mice[20] with the MLC2v Cre deleter line[21] to test *Zbtb17* gene function in cardiac myocyte–specific cKO (Figure 3A–3D in the Data Supplement). cKO mice were viable and, in the absence of a spontaneous functional cardiac phenotype, we used the established model of transverse aortic constriction (TAC)[5] to unveil potential gene effects. After TAC, the left ventricles of cKO mice became dilated and exhibited impaired contractile performance, which are the cardinal features of DCM (Table 1). When compared with controls, failing cKO hearts had higher apoptotic events (8.5-fold; $P<0.001$), increased activated caspase 3 (6.3-fold; $P<0.001$), and marked replacement fibrosis (Figure 4A; Figure IIIE and Table II in the Data Supplement). These data show that Zbtb17 modulates biomechanical stress–induced apoptosis and interstitial fibrosis in vivo, which are important in the pathobiology of both HCM and DCM.

To begin to understand the molecular mechanisms by which ZBTB17 regulates cardiac myocyte survival, based on the functions of ZBTB17 in other tissues,[19] we combined our microarray data with apoptosis-specific gene expression array analysis (quantitative real-time polymerase chain reaction based, n=84) in cKO.TAC and control.TAC mouse hearts. We used Gene Set Enrichment Analysis[22] to test whether differentially expressed genes by genome-wide microarray analysis were significantly enriched in the set of differentially expressed genes detected by apoptosis-specific quantitative real-time polymerase chain reaction array analysis and found significant concordance for a set of 8 genes ($P<0.05$, false discovery rate, <1%; Table 2). Among these, the proapoptotic genes were consistently upregulated in cKO, whereas the antiapoptotic genes, Nol3 and Tnfrsf1a, were downregulated in cKO hearts after biomechanical stress, supporting a role for Zbtb17 in the regulation of apoptosis. Six of 8 differentially expressed genes were also detected by ChIP-chip analysis to have binding sites (Table 2). Hence ZBTB17 regulates a transcriptional program that protects cells against apoptosis.

In addition, ZBTB17 overexpression drives hypertrophy in vitro (Figure 3). To examine further the effects of *ZBTB17* on hypertrophy we overexpressed *ZBTB17* in mice using a cardiac myocyte–specific promoter[23] (Figure IV in the Data Supplement). Transgenic mice (TG-ZBTB17) exhibited spontaneous cardiac hypertrophy with enlarged cardiac myocytes and increased Nppa expression (Table I in the Data Supplement ; Figure 4B and 4C). In vitro experiments suggested a link between ZBTB17 and calcineurin, and ChIP-chip data showed binding of ZBTB17 to the calcineurin Aβ (Ppp3cb) promoter that we confirmed by ChIP quantitative real-time polymerase chain reaction and reporter assays (Figure 4D and 4E). There was increased expression of Ppp3cb and Nfatc2 in TG-ZBTB17 hearts and decreased expression in cKO (Figure 4F; Figure VA in the Data Supplement). We crossed *ZBTB17* transgenic mice into a Ppp3cb-deficient

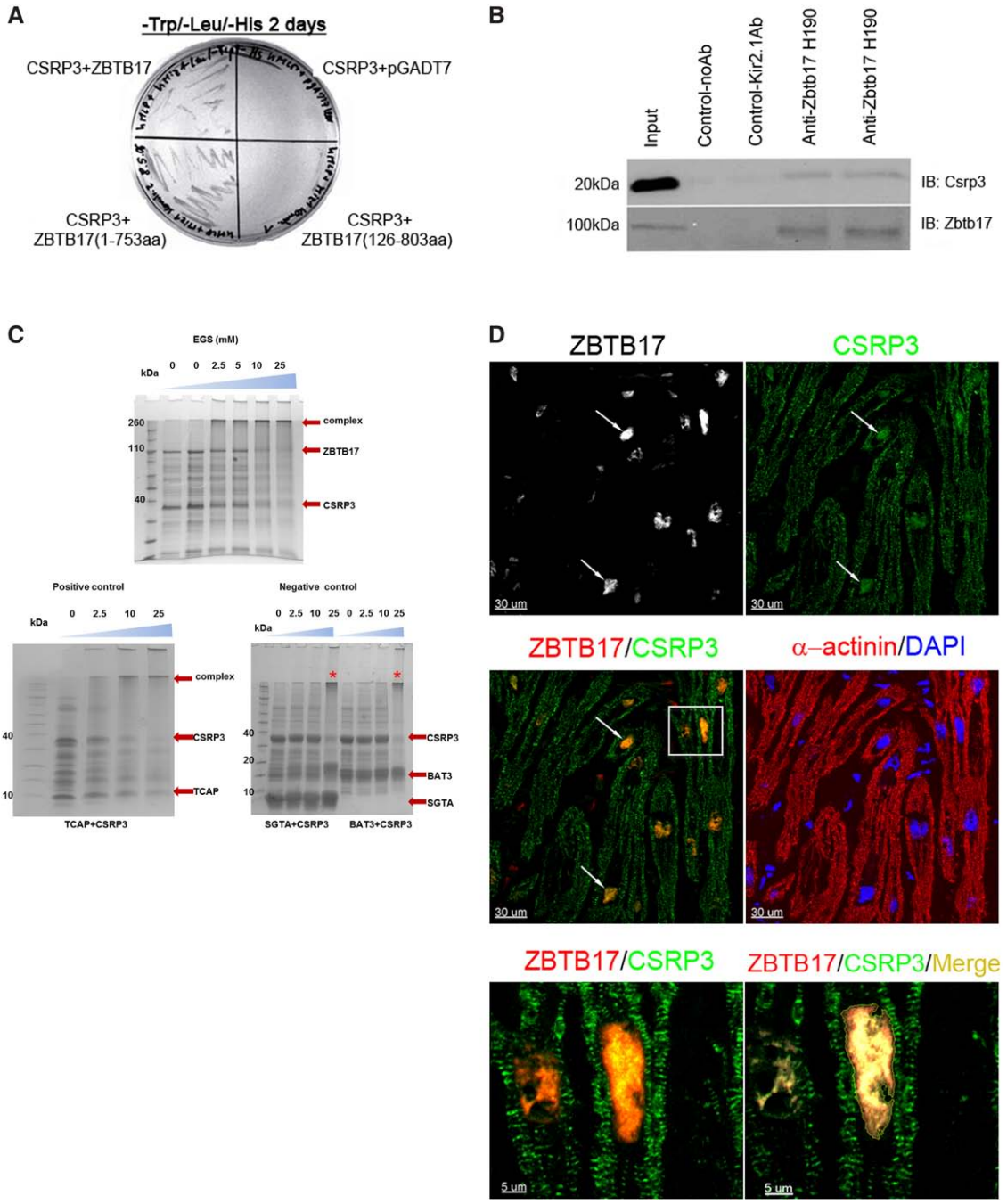

Figure 1. **A**, Analysis of the interaction between cysteine and glycine-rich protein 3 (CSRP3) and zinc-finger and BTB domain-containing protein 17 (ZBTB17) by yeast 2-hybrid assays. No interaction occurs between proteins when the ZBTB17-POZ domain is deleted (lower right quadrant). A total of 10^6 clones were screened, and 650 positive clones were identified,[3] one of which encoded the 5′ sequence of *Zbtb17*. **B**, Immunoprecipitation of ZBTB17 from heart lysates results in the coprecipitation of CSRP3. Input indicates mouse heart extract, a Csrp3 antibody (sc-30274; Santa Cruz Biotechnology) has been used for immunoprecipitation, and a well-characterized Zbtb17 (Santa Cruz H-190; sc 22837) antibody has been used to detect the protein. No antibody or an unrelated anti-Kir2.1 antibody served as negative controls. **C**, Cross-linking experiments of recombinant proteins confirm binding between ZBTB17 and CSRP3. **Top**, With increasing concentration of cross-linking reagent, the individual components decrease, whereas a large complex emerges. The complex contains multiple copies of each protein reflecting the oligomeric nature of the individual components. **Bottom left**, Positive control showing cross-linking between CSRP3 and Telethonin (TCAP), a known interaction. **Bottom right**, Negative controls: we attempted to cross-link CSRP3 separately with 2 different proteins that have no documented association but are known to cross-link well to their binding proteins. These were small glutamine-rich tetratricopeptide repeat-containing protein alpha (SGTA) and HLA-B associated transcript 3 (BAT3). As expected, increasing cross-linking reagents showed no binding between CSRP3 and the negative control proteins, but only the usual CSRP3 oligomers indicated with stars. **D**, Colocalization of ZBTB17 and CSRP3 in the nuclei of human cardiac myocytes. Arrows indicate typical cardiac myocyte nuclei that are positive for both ZBTB17 and CSRP3. **Lower left**, Three-dimensional image of the boxed region (**left middle**). **Lower right**, Three-dimensional colocalized color intensity image of ZBTB17 with CSRP3: mean percentage of nuclear ZBTB17 colocalized with nuclear CSRP3=79.4±5.5%; mean Pearson coefficient of colocalized volumes=0.88 (n=4, ≈125 nuclei per heart).

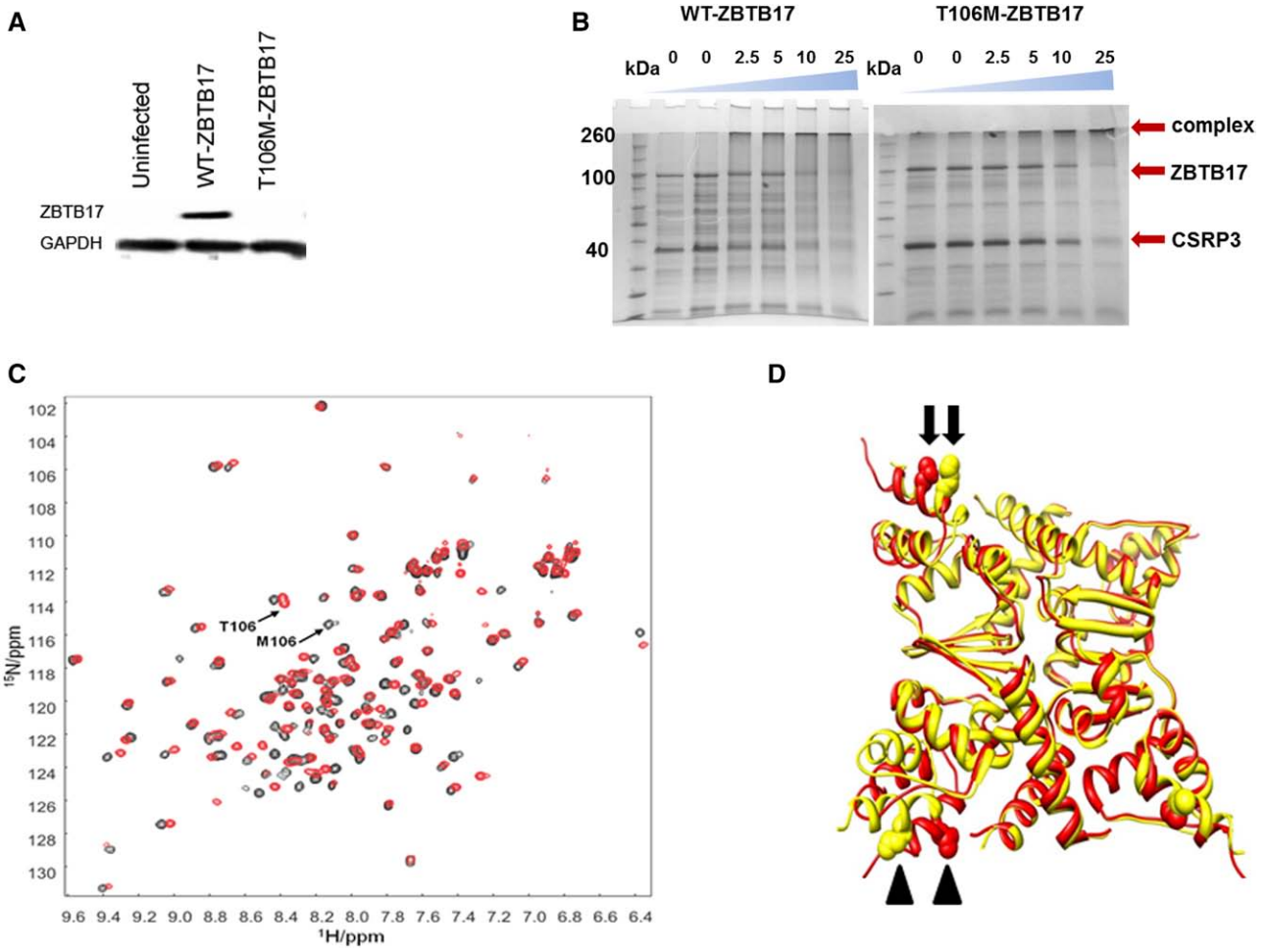

Figure 2. **A**, Analysis of wild-type (WT)- and T106M–zinc-finger and BTB domain-containing protein 17 (ZBTB17) proteins via adenoviral overexpression in neonatal rat cardiac myocytes. Although WT-ZBTB17 protein is readily detectable, ZBTB17:p.T106M was not and is most likely unstable in eukaryotic cells. **B**, Interaction of T106M-ZBTB17 with cysteine and glycine-rich protein 3 (CSRP3) as studied via cross-link interaction. CSRP3 interacts with WT-ZBTB17 (**left**), but interacts less well with T106M-ZBTB17 (**right**). **C**, NMR1H-$^{15\,N}$ HSQC spectrum of WT (red) and T106M (black) ZBTB17-POZ domain with pertinent residues assigned based on predicted chemical shift. The mutation causes significant structural disruption to the POZ domain. **D**, The available ZBTB17-POZ structure was used to model the T106M mutation. Fifty nanoseconds of simulation of the WT and the T106M structures that have been run as the full quadrameric structures as in the PDB 2Q81. Red indicates the native and yellow the mutant ZBTB17, spheres represent the T and M residues. It can be clearly seen that the structures of the α-6, where the mutation is located, have changed. Also the simulations reveal that there is a much higher level of structural mobility for the mutant case. The mutation causes significant structural disruption to the POZ domain, a finding consistent with our NMR data, arrowheads indicate position 106, arrows indicate a position far away).

background that prevented cardiac myocyte hypertrophy and decreased Nppa expression, confirming the interaction of ZBTB17 with the calcineurin pathway (Figure 4B; Table I in the Data Supplement).

Discussion

CSRP3 mutations cause heart failure in various animal models[4,24] and have been identified in various patients affected by DCM[3,25] or HCM.[26–28] Although many cardiomyopathy and heart failure models have been developed, the molecular mechanisms that link sarcomeric and Z disc proteins to these phenotypes remain not well understood. Here, we show that CSRP3 interacts with ZBTB17 and provide the first detailed analysis of this transcription factor in the cardiovascular system. In particular, we demonstrate that *ZBTB17* causes cardiac myocyte hypertrophy and is essential for cell survival. The extent of hypertrophy in cardiac myocytes is comparable with the effects seen with trophic signals including mitogenic serum in these postmitotic cells.[29,30] To date, ZBTB17 is the only known transcription factor to interact with CSRP3 and to be expressed in cardiac myocytes.[31] The effects of ZBTB17 on hypertrophy and survival are not only restricted to the in vitro situation but also observed in genetically altered animals under in vivo conditions. Interestingly, ZBTB17-overexpressing transgenic animals did not develop heart failure because of massive increase in apoptosis as frequently seen in other genetically altered mouse models.[32–35]

The combination of ChIP-chip, whole-gene expression, and apoptosis array data clearly point to an important role of ZBTB17 in orchestrating a cardioprotective gene expression

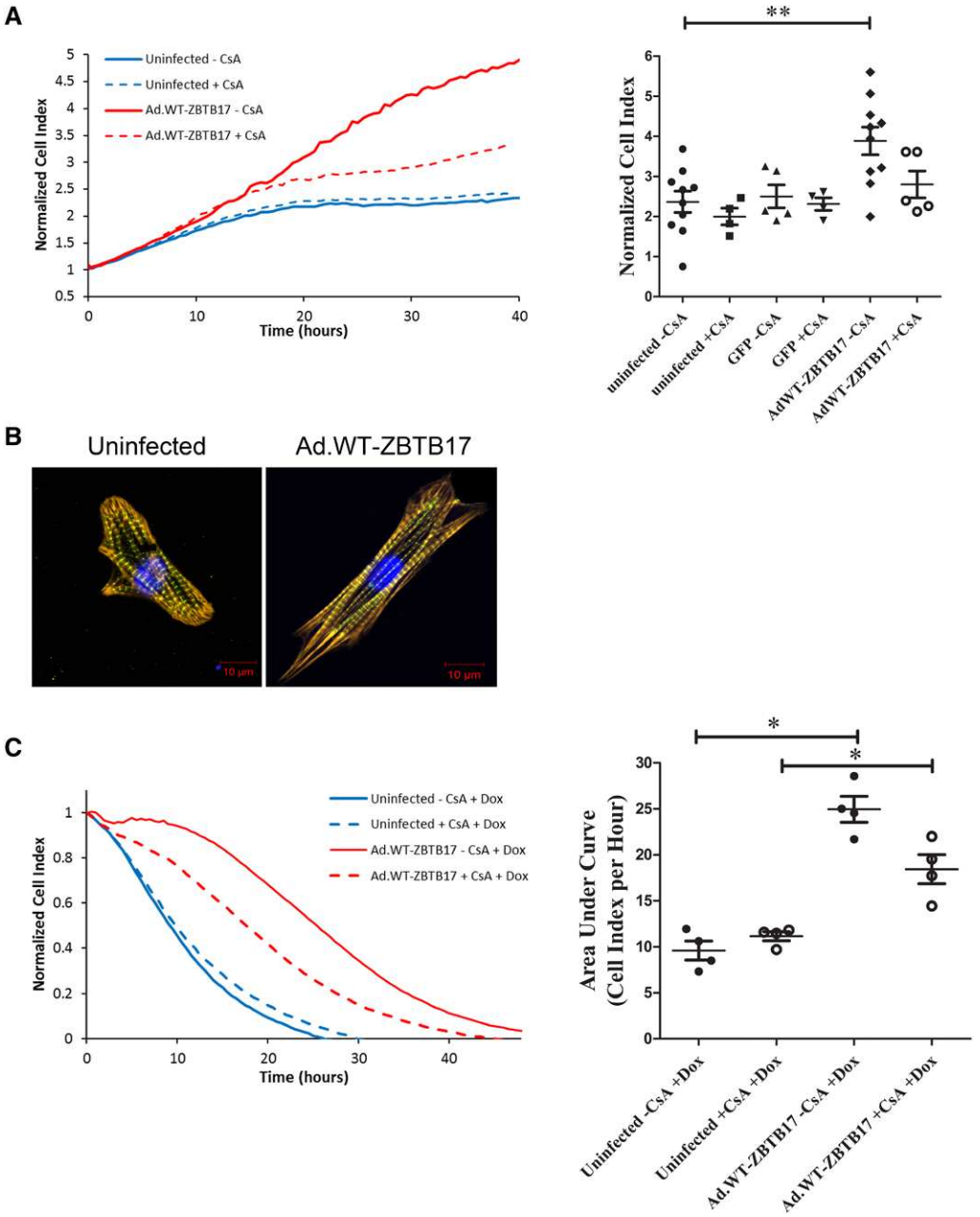

Figure 3. A, Analysis of zinc-finger and BTB domain-containing protein 17 (ZBTB17) overexpression in neonatal rat cardiac myocytes (NRCMs) as determined by impedance measurements. Scatter dot plot represents cell index values after 24 h (n=10, experiments done in duplicate). Dotted lines indicate cell indices in calcineurin inhibition experiments (Cyclosporin A [CsA]: 0.2 µmol/L; n=4; Mann–Whitney *U* test was used for the comparison of groups, $^{**}P<0.01$). **B**, Representative images of NRCMs with and without ZBTB17 adenoviral gene transfer. **C**, Effects of *ZBTB17* overexpression on doxorubicin (DOX, 1 µmol/L)–induced cell death (n=4). Dotted lines indicate data from calcineurin inhibition experiments (CsA: 0.2 µmol/L; n=4; Mann–Whitney *U* test was used for the comparison of groups, $^{*}P<0.05$).

program. This is indicated by the fact that proapoptotic genes were consistently upregulated, whereas antiapoptotic genes were downregulated in cKO hearts after biomechanical stress (Table 3).

We also show that ZBTB17 targets various calcineurin/NFAT genes and activates this pathway. This notion is supported by our whole-gene expression analysis, ChIP-chip assays, analysis of the calcineurin/NFAT pathway activation, promoter assays, and by the significant loss of hypertrophy when TG-ZBTB17 animals are crossed into the calcineurin *A*β–deficient background (Figures 3 and 4D and 4E). These data are also supported by our finding of enhanced calcineurin *A*β and *Nfatc2* mRNAs in hearts of *ZBTB17* transgenic animals and decreased calcineurin *A*β and *Nfatc2* mRNAs in hearts of *Zbtb17* cKO animals after TAC (Figure 4F; Figure VA in the Data Supplement).

CSRP3 translocates into the nucleus on TAC[36] and is essential for the adaptation of cardiac myocytes to biomechanical stress.[37] CSRP3, like telethonin, is primarily expressed in muscle tissues. By interacting with ZBTB17, CSRP3 plays a

Table 1. Echocardiography of cKO-*Zbtb17* Animals

Genotype/ Intervention	BW, g	LVIDd, mm	LVIDs, mm	FS%	h/r	HW/BW, mg/g	$LV_{Vol}s$	$LV_{Vol}d$	EF%
Control.SHAM	31.90	3.77	2.06	45.61	0.54	4.26	14.40	61.39	45.62
Control.TAC	31.01	3.44*	1.65*	52.01*	0.63*	5.03*	7.86*	49.04*	52.04*
cKO.SHAM	32.04	3.71	2.05	45.71	0.56	4.07	15.30	59.79	45.73
cKO.TAC	32.55	3.74‡	2.00†	46.46†	0.57	4.82	13.22†	59.69†	46.46†

Functional data obtained via echocardiography 4 weeks after sham (SHAM) and transverse aortic constriction (TAC) operations in *Zbtb17* conditional knockout (cKO) and control animals. After TAC, cKO hearts enlarge (LVIDd and LVIDs) and decrease in function (FS%; control=flox/flox, Cre^- and cKO=flox/flox, Cre^+). BW indicates body weight; EF, ejection fraction; h/r, left ventricular wall thickness divided by left ventricular radius; HW, heart weight; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; $LV_{Vol}d$, left ventricular diastolic volume; and $LV_{Vol}s$, left ventricular systolic volume.

*$P<0.05$, for control.SHAM vs control.TAC.

†$P<0.05$.

‡$P<0.01$ for control.TAC vs cKO.TAC; n=6 per group. Student *t* test was used for the comparison of groups.

role in the initiation of myocyte-specific survival pathways or mechanoptosis (mechanosensitive types of cell death[5]). This notion is supported by the fact that *Csrp3*-deficient hearts exhibit increased rates of apoptosis,[38] hence the interaction of CSRP3 with ZBTB17 can also be described as a form of Z disc transcriptional coupling.[39]

Calcineurin/NFAT signaling is an important mediator of cardiac hypertrophy,[40,41] and this pathway is also known to exert cardioprotective effects by avoiding apoptosis during ischemia reperfusion,[15] which emphasizes the effects of ZBTB17 on cell survival.

CSRP3 directly interacts with calcineurin and is required for its activation,[42] thus by interacting with ZBTB17; CSRP3 introduces an additional level of control, which ensures an intermediate level of calcineurin/NFAT activation (Figure 4G). For a long time, c-myc is known to be induced during TAC,[43] but its downstream effects in cardiac myocytes remained elusive. However, c-myc is known to inhibit ZBTB17[44] and therefore may have adverse effects on survival and hypertrophy in cardiac myocytes. Interestingly, CSRP3 interacts with the aminoterminal POZ domain, which is predicted to interfere with ZBTB17 tetramerization.

An additional layer of complexity is added by the fact that ZBTB17 can be phosphorylated at position S428 by the Akt kinase, which is another important mediator of hypertrophy.[45] This post-translational modification enables the interaction of ZBTB17 with 14-3-3 ŋ and leads to its inactivation, thus avoiding prolonged activation of ZBTB17, which can also have adverse effects.[46]

In the Exome Variant Server database, there are 10 T106M variants annotated out of at least 13 006 alleles of European-American and Afro-American origin combined. The Exome Variant Server does not provide data on individual phenotypes, but the frequency for this variant is certainly low and a disease causing or at least modifying role cannot be excluded. Nevertheless, a 2-hit hypothesis whereby a structural perturbation combines with a defect in a ZBTB17-mediated cell survival pathway could also lead to heart failure.

As a result, we expect this variant to impair calcineurin-dependent hypertrophy and being unable to efficiently protect cardiac myocytes from apoptosis. However, this variant may also have specific effects on remodeling and hypertrophy[47] by specifically affecting protein degradation pathways, for example, via the ubiquitin proteasome system,[48] and aside from affecting its interaction with CSRP3 (Figure 2B), may specifically interfere with other protein/protein interactions.

ZBTB17 is a transcription factor that contains the peak association signal (rs10927875) at the replicated 1p36 cardiomyopathy locus. Although other genes in the genomic region of *ZBTB17*, such as *HSPB7* and *CLCNKA*, have been suggested as possible candidate genes by genome-wide association study,[10,49] *ZBTB17* might well be another novel DCM-associated gene. However, except for this report, the effects of ZBTB17 on the cardiovascular system have never been analyzed before. It is interesting to note that *HSPB7*, which encodes heat shock protein 27, a factor involved in the immediate stress response, *CLCNKA*, which encodes a K_a renal chloride channel involved in the regulation of hypo-osmotic cell stretch, as well as *ZBTB17*, are all involved in the cellular primary stress response and survival signaling.

At least 50 single genes have been identified as linked to familial DCM, some of which encode proteins of the sarcomere,[50] costamere, Z disc,[3,4] and nuclear membrane,[51] whereas others function as phosphatases and transcriptional activators (EYA4).[1,52] Unfortunately, not much is known about how these effects are linked to changes in gene transcription. A recent study found profibrotic gene expression profiles in HCM mouse models and identified transforming growth factor beta 1 (*TGFβ1)* as the initiating event, but the underlying transcriptional events remained elusive.[53] However, ZBTB17 may well play a role in these genetic circuits. Defective Z disc–mediated survival signaling may also contribute to the DCM phenotype observed in patients carrying truncating Titin (*TTN)* mutations.[50]

In summary, we identify *ZBTB17* as a candidate for a new cardiomyopathy gene, which may also be important for heart failure syndromes in general, and suggest that its primary function is to protect cardiac myocytes from apoptosis[5] through modulation of both hypertrophic and cell death pathways (Figure 4G).

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Dr Molkentin is kindly acknowledged for providing the calcineurin Aβ Promotor-Luciferase plasmid and calcineurin Aβ

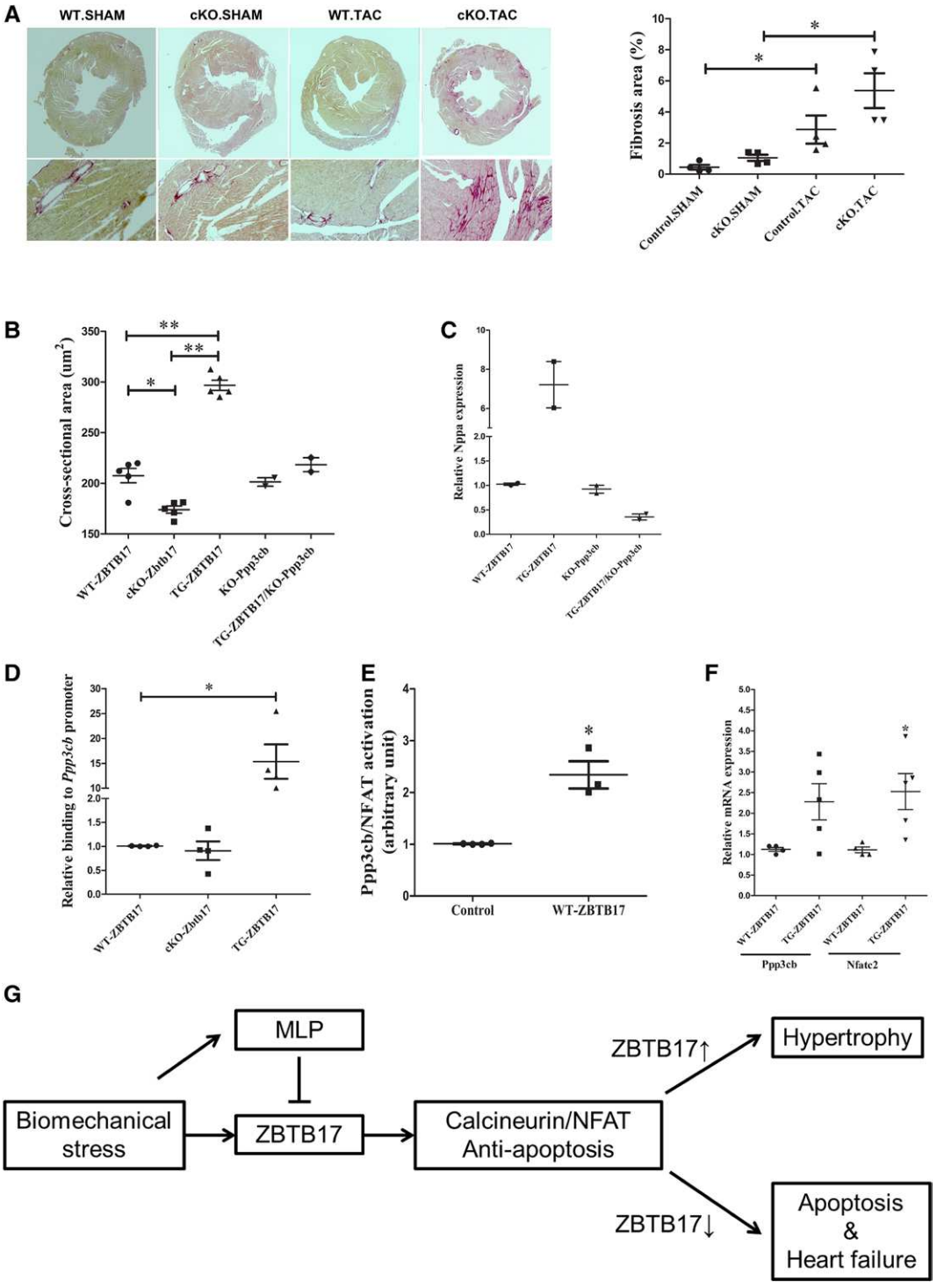

Figure 4. **A**, Representative micrographs of fibrosis staining of mouse hearts after sham (SHAM) or transverse aortic constriction (TAC) procedures and quantification of fibrosis (4 data points per animal, 1 representative experiment of 2 shown; Mann–Whitney test was used for the comparison of groups; **P*<0.05). **B**, Analysis of cardiac myocyte cross-sectional area under spontaneous conditions in wild-type (WT)-zinc-finger and BTB domain-containing protein 17 (ZBTB17), conditional knockout (cKO)-Zbtb17, transgene (TG)-ZBTB17 (≈150 cardiac myocytes per animal; n=5 per group, Mann–Whitney *U* test was used for the comparison of groups, **P*<0.05, ***P*<0.01), and KO-Ppp3cb, TG-ZBTB17/KO-Ppp3cb (≈150 cardiac myocytes per animal; n=2 per group). **C**, Upregulation of the prohypertrophic biomarker Nppa in TG-ZBTB17 hearts (n=2 per group, experiments done in duplicate). **D**, Chromatin immunoprecipitation microarray quantitative real-time polymerase chain reaction analysis of Zbtb17 binding to the Ppp3cb promoter in vivo (1 representative experiment of 2 shown, Mann–Whitney *U* test was used for the comparison of groups, **P*<0.05). **E**, Luciferase reporter assay of ZBTB17 activation of the Ppp3cb promoter via transient transfection (n=3–4 per group, Mann–Whitney *U* test was used for the comparison of groups, **P*<0.05). **F**, Increased *Ppp3cb* and *Nfatc2* mRNA expression levels in TG-ZBTB17 (1 representative experiment of 2 shown, Mann–Whitney *U* test was used for the comparison of groups, **P*<0.05). **G**, ZBTB17 is important for the adaptation of cardiac myocytes to biomechanical stress. Loss of ZBTB17 is associated with an increase in cardiac myocyte apoptosis and heart failure, whereas overexpression leads to activation of the calcineurin/nuclear factor of activated T-cell (NFAT) pathway and cardiac myocyte hypertrophy. MLP indicates muscle LIM protein or CSRP3.

Table 2. Summarized Expression Profiles and Zbtb17 Binding Sites of Target Genes Involved in Cardiac Myocyte–Specific Programmed Cell Death in cKO Animals (n=6 Per Group)

Apoptosis RT² Profiler PCR Array data (SA Biosciences)					Whole-transcript array data (Affymetrix Microarray)			GSEA	ChIP-Chip
Gene	Control TAC	cKO TAC	Fold Change	*P* Value	Control TAC	cKO TAC	Fold Change	Contribution to Gene Enrichment	FDR Value of Binding
Casp1	0.0034	0.006	1.76	0.028	0.0193	0.0386	2	+	0.00214
Casp2	0.0098	0.0215	2.2	0.002	0.0341	0.0431	1.26	+	0.02509
Casp3	0.003	0.0044	1.5	0.062	0.0223	0.0583	2.61	+	0.05001
Casp9	0.0112	0.0207	1.86	0.057	–0.0216	0.0107	2.5	+	0.01765
Traf3	0.0044	0.0071	1.61	0.008	–0.057	–0.0105	1.82	+	0.04486
Casp12	0.0008	0.0015	1.84	0.085	0.069	0.1771	2.57	…	0.02754
Nol3	0.0282	0.0198	0.7	0.008	0.1438	0.0985	0.68	…	NBS
Tnfrsf1a	0.0157	0.0092	0.59	0.054	0.1712	–0.0728	–0.43	…	NBS

Directional change of genes is consistent with the antiapoptotic role of endogenous Zbtb17. cKO indicates conditional knockout; GSEA, Gene Set Enrichment Analysis; PCR, polymerase chain reaction; NBS, no ZBTB17 binding site; TAC, transverse aortic constriction.

(Ppp3cb)–deficient animals. Dr Wright is thanked for providing the ZBTB17-POZ plasmid.

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Disclosures

None.

Appendix

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Table 3. In Vivo Analysis of TUNEL-Positive Cells, Activated Caspase 3, and Collagen I

	TUNEL (n/1000 Cardiomyocytes)		Activated Caspase 3 (n/1000 Cardiomyocytes)		Collagen I (% Tissue Area)	
Animals	Control.TAC	cKO.TAC	Control.TAC	cKO.TAC	Control.TAC	cKO.TAC
1	1.04	11.35	3.53	17.73	5.89	10.71
2	1.73	12.55	3.86	21.81	6.63	12.53
3	1.49	9.41	4.01	19.55	7.22	16.24
4	2.38	15.44	3.37	19.92	5.14	11.25
5	0.95	13.87	3.88	25.77	7.73	15.02
6	1.72	8.08	1.85	23.19	6.85	13.09
Mean±SD	1.55±0.5	11.78±2.5*	3.42±0.73	21.32±2.63*	6.57±0.85	13.29±1.98*

cKO indicates conditional knockout; RT2, real-time-2 referring quantitative real-time PCR; TAC, transverse aortic constriction; and TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

Student *t* tests were used for the comparison of groups, data=mean±SD; Control.TAC: flox/flox, Cre^{-}; cKO.TAC: flox/flox, Cre^{+}; n=6 per group. *$P<0.001$.

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CLINICAL PERSPECTIVE

Mutations in >50 genes, mostly encoding sarcomeric and Z disc proteins, are a major cause of hereditary cardiomyopathies. However, most likely this list is incomplete, and, probably most importantly, the underlying molecular disease-causing mechanisms remain not well defined. Here, we used cysteine and glycine-rich protein 3 (also known as muscle LIM protein), a known cardiomyopathy gene, and identified zinc finger and BTB domain-containing protein 17 (*ZBTB17*) as a novel interacting partner. *ZBTB17* is a transcription factor that contains the peak association signal (rs10927875) at the replicated 1p36 cardiomyopathy locus, identified in various recent genome-wide association studies. Loss of *Zbtb17* in cardiac myocytes causes apoptosis and heart failure, whereas overexpression of *ZBTB17* drives calcineurin/nuclear factor of activated T-cell (NFAT)–dependent cardiac myocyte hypertrophy. Here, we provide the first detailed analysis of *ZBTB17* in the cardiovascular system and provide evidence for ZBTB17 being a novel cardiomyopathy candidate gene. ZBTB17 functions might be exploited for the development of innovative therapies to treat heart failure, an otherwise lethal condition.